

THERAPEUTIC METHOD

This invention is directed to new therapeutic uses which involve the stimulation of chondrocyte proliferation. More particularly, it is directed to the use of amylin and adrenomedullin as agents which stimulate chondrocyte proliferation and which therefore have utility in the treatment of cartilage disorders and/or cartilage mediated bone growth.

**BACKGROUND**

Amylin is a 37-amino acid peptide cosecreted with insulin from the beta cells of the pancreatic islets. It was first reported by Cooper *et al* in Proceedings of the National Academy of Sciences, USA 84, 8628 (1987) and is the subject of European Patent 289287. Amylin has the following peptide sequence:

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-

1 5 10

Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-

11 15 20

Asn-Asn-Phe-Gly-Ala-Ile-Leu-Ser-Ser-Thr-

21 25 20

Asn-Val-Gly-Ser-Asn-Thr-Tyr

31 35

The native molecule contains a disulphide bridge between the cysteine residues shown at positions 2 and 7 in the primary structure, is amidated at its COOH-terminus, and is formed as a propeptide.

European Patent 289287 reports a number of biological effects including enhancement of hepatic glucose output, increased production of lactate from skeletal muscle and reduced action of insulin in skeletal muscle.

Amylin is also reported in European Patent 408284 as having value for treatment of bone disorders and calcium imbalance. The patent specification attributes the activity of amylin to an inhibition of osteoclast motility. It is also reported in WO 96/02269 as stimulating bone growth through stimulating osteoblast proliferation.

Adrenomedullin is a 52-amino acid peptide first described in 1993 by Kitamura *et al* (Kitamura, K., *et al.* Adrenomedullin, a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem. Biophys. Res. Commun.* 192:553-560 (1993)). It is present in normal adrenal/medulla and in many other tissues including the atria, ventricles, endothelial cells, lungs, brain, kidneys and bone.

Adrenomedullin shows approximately 20% sequence identity with amylin and can therefore be termed a related peptide (Muff, R., *et al.* Calcitonin, calcitonin gene-related peptide, adrenomedullin and amylin: homologous peptides, separate receptors and overlapping biological actions. *Eur. J. Endocrinol.* 133:17-20 (1995)). Both peptides have an NH<sub>2</sub> terminal ring created by a disulphide bond and are amidated at their COOH terminals.

Like amylin, adrenomedullin is also reported to have a range of activities. It is a potent vasodilator. It also has value in the treatment of bone disorders. This is primarily through an ability to stimulate osteoblast activity and proliferation *in vitro* and *in vivo* (Cornish, J., *et al.* Adrenomedullin is a potent stimulator of osteoblastic activity *in vitro* and *in vivo*. *Am. J. Physiol (Endocrinol Metab)* 36:E1113-E1120, (1997)).

However, to date, there has been no report of either of the peptides amylin or adrenomedullin, as having any effect on chondrocytes. It is the applicants finding that both of these peptides are effective in the stimulation of chondrocyte proliferation and therefore on the growth of both cartilage and lineal bone. This effect is believed to be mediated through a single receptor on chondrocytes which underlies the applicant's invention.

## SUMMARY OF THE INVENTION

The invention has a number of aspects. In a first aspect, the invention provides a method of treating a patient to stimulate chondrocyte proliferation *in vivo* which comprises the step of increasing the active concentration of amylin within said patient.

Another aspect provides a method of treating a patient to stimulate chondrocyte proliferation *in vivo* which comprises the step of administering to said patient amylin or an analog thereof in an amount effective to stimulate chondrocyte proliferation.

In another embodiment, the invention provides a method of treating a patient to stimulate chondrocyte proliferation *in vivo* which comprises the step of increasing the active concentration of adrenomedullin within said patient.

In a further embodiment, the invention provides a method of treating a patient to stimulate chondrocyte proliferation *in vivo* which comprises the step of administering to said patient adrenomedullin or an analog thereof in an amount effective to stimulate chondrocyte proliferation.

In still a further aspect, the invention provides a method of treating a patient to stimulate chondrocyte proliferation *in vivo* which comprises the step of activating the receptor localised on chondrocytes of said patient to which amylin and/or adrenomedullin bind.

Most preferably, the method involves activation of the adrenomedullin receptor.

Conveniently, in each of the above methods the stimulation of chondrocyte proliferation is part of a method of treating a patient to stimulate cartilage growth and/or repair or to stimulate bone growth.

The invention also provides a method of stimulating chondrocyte proliferation *in vitro* which comprises administering an amount of amylin, adrenomedullin or an analog of either amylin or adrenomedullin to said chondrocytes which is effective in inducing chondrocyte proliferation.

Other aspects include:

- INS  
A.2
- 5 the use of amylin or an analog thereof in the preparation of a medicament for effecting chondrocyte proliferation;
- the use of adrenomedullin or an analog thereof in the preparation of a medicament for effecting chondrocyte proliferation;
- 10 the use of a ligand which binds to and activates the receptor to which amylin and/or adrenomedullin binds (preferably the adrenomedullin receptor) in the preparation of a medicament for effecting chondrocyte proliferation;
- 15 the use of an amylin agonist in the preparation of a medicament for effecting chondrocyte proliferation;
- the use of an adrenomedullin agonist in the preparation of a medicament for effecting chondrocyte proliferation;
- 20 the use of amylin-(1-8) in the preparation of a medicament for effecting chondrocyte proliferation; and
- 25 the use of adrenomedullin-(27-52) in the preparation of a medicament for effecting chondrocyte proliferation.

#### DESCRIPTION OF THE DRAWINGS

30 The present invention is broadly as defined above. However, it will be appreciated by those persons skilled in the art that it is not limited thereto and that it also includes embodiments which are more particularly described below and illustrated by the experimental data presented. This data includes the information shown in the accompanying drawings in which:

Figure 1 shows the effects of daily systemic administration of amylin for 4 weeks on growth plate width in the tibiae of normal adult male mice.  $n = 20$  in each group. \*, significantly different from control,  $P = 0.0002$ ;

- 5 Figure 2 shows the effects of daily systemic administration of amylin for 4 weeks on bone length in the tibiae of normal adult male mice.  $n = 20$  in each group. \*, significantly different from control,  $P = 0.004$ ;

- 10 Figure 3 shows the effect of the amylin fragment (amylin (1-8)) on epiphyseal growth plate width; and

Figure 4 shows the effect of the adrenomedullin fragment (adm 27-52) on epiphyseal growth plate width.

15 **DESCRIPTION OF THE INVENTION**

As broadly defined above, the present invention relates primarily to methods for stimulating chondrocyte proliferation. The invention therefore has utility in any application where stimulation of chondrocyte proliferation or growth is viewed as  
20 desirable, including for example cartilage growth and bone growth.

- The applicants have found that chondrocyte proliferation is able to be effected using a number of related approaches. A first such approach is through a focus upon amylin. The applicants have found that increasing the effective concentration of  
25 amylin within a patient able to interact with the patients chondrocytes has the effect of stimulating chondrocyte proliferation.

Amylin for use in accordance with this approach can be obtained from any convenient commercial source (such as Bachem California, Torrence, CA, USA).

- 30 Alternatively, amylin can be synthesised, using the procedure as described by way of example in EP 408284.

- The amylin used can be homologous or heterologous to the patient to be treated. For example, amylin from humans and other mammals eg. rat, monkey, dog, cat,  
35 mouse, guinea pig, hamster, degus, rabbit and hare can be used. The structure of

these various peptides is reported in *Endocrine Reviews* 1994, 15(2) 163 by Garth J S Cooper which is incorporated herein by reference.

5 Most conveniently, the effective concentration of amylin will be increased through direct administration using either amylin itself or an amylin pro-drug (a form which is cleaved within the body to release amylin). It is however not the applicants intention to exclude increasing amylin concentration through administration of either amylin agonists (substances which effect a direct increase in the production or activity of amylin within the body, or inhibitors of amylin antagonists (substances  
10 which bind amylin or otherwise prevent or reduce the action of amylin within the body. These latter compounds exert an indirect effect on effective amylin concentrations through the removal of an inhibitory mechanism.

15 Another possibility is administration of a replicable vehicle encoding amylin to the patient. Such a vehicle (which may be a modified cell line or virus which expresses amylin within the patient) could have application in increasing the concentration of amylin within the patient for a prolonged period.

20 It is also contemplated that amylin analogs can be employed in this invention. As used herein "analog" means a protein which is a variant of another protein through insertion, deletion or substitution of one or more amino acids but which retains at least substantial functional equivalency.

25 A protein is a functional equivalent of another protein for a specific function if the equivalent protein is immunologically cross-reactive with, and has at least substantially the same function as, the original protein. The equivalent can be, for example, a fragment of the protein, a fusion of the protein with another protein or carrier, or a fusion of a fragment with additional amino acids. For example, it is possible to substitute amino acid in a sequence with equivalent amino acids using  
30 conventional techniques. Groups of amino acids normally held to be equivalent are:

(a) Ala, Ser, Thr, Pro, Gly;

(b) Asn, Asp, Glu, Gln;

(c) His, Arg, Lys;

35 (d) Met, Leu, Ile, Val; and

(e) Phe, Tyr, Trp.

5 In the case of amylin, the preferred analogs are fragments of the protein. In particular, amylin (1-8) can be used (ie. a fragment consisting of amino acids 1 to 8 of the amylin sequence).

Functional equivalency of analogs can also be readily screened for by reference to the ability of the analog to both bind to and activate the appropriate receptor.

10 In addition to the above approach, which focuses upon amylin and its analogs, the invention provides a further approach to chondrocyte proliferation. This second approach has a focus upon adrenomedullin. The applicants have found that, in an equivalent manner to amylin, increasing the effective concentration of adrenomedullin within a patient able to interact with the chondrocytes in that  
15 patient stimulates chondrocyte proliferation.

For use in this approach, adrenomedullin can be obtained from any convenient commercial source or, as is the case with amylin, synthesised using techniques well known in the art. Such techniques include those described hereinafter.

20 Again, it is most convenient that the effective concentration of adrenomedullin be increased through direct administration using either adrenomedullin itself or an adrenomedullin pro-drug. However, adrenomedullin agonists or inhibitors of adrenomedullin antagonists are not excluded.

25 As with amylin, adrenomedullin can also be administered in the form of a replicable vehicle encoding adrenomedullin to the patient for release of adrenomedullin over a prolonged period.

30 Adrenomedullin analogs can also be employed. For this purpose, the term "analog" has the equivalent meaning of that given above for amylin. In the case of adrenomedullin, a particularly preferred analog is adrenomedullin (27-52) (ie. a fragment consisting of amino acids 27-52 of the adrenomedullin sequence).

35 The invention still further provides a third approach to chondrocyte proliferation. This further approach focuses upon the receptors on chondrocytes to which amylin

and adrenomedullin bind and upon effecting chondrocyte proliferation through use of any ligand which both binds to and activates these receptors.

It will be appreciated that amylin, analogs of amylin, adrenomedullin and analogs of  
5 adrenomedullin are all ligands which achieve this. Indeed, the use of these substances as active agents represents a preferred aspect of the invention. However, it should be appreciated that this approach has not restricted the use of amylin, adrenomedullin and their analogs but also extends to any ligand which fulfils the functional requirement of both binding to and activating (stimulating) the  
10 amylin or adrenomedullin receptors. Such additional ligands are, for example, believed to include peptides such as calcitonin gene related peptide (Muff, R., *et al.* Calcitonin, calcitonin gene-related peptide, adrenomedullin and amylin: homologous peptides, separate receptors and overlapping biological actions. *Eur. J. Endocrinol.* 133:17-20 (1995)).

15 A specific feature of this approach is to employ ligands which bind to and activate the adrenomedullin receptor. This receptor was described in, for example, Kapas, S., *et al.* Cloning and expression of cDNA encoding a rat adrenomedullin receptor. *J. Biol. Chem.* 270:25344-25347 (1995). It is further described in Montuenga, L. M.,  
20 *et al.* Expression of adrenomedullin and its receptor during embryogenesis suggests autocrine or paracrine modes of action. *Endocrinology* 138:440-451 (1997)).

Additional stimulatory ligands can therefore, for example, be identified by a screening protocol employing at least the ligand binding domain of the  
25 adrenomedullin receptor. This screening method can, for example, utilise the expression of the adrenomedullin receptor in *Xenopus* oocytes using standard recombinant DNA methods and measurement of the adrenomedullin receptor-mediated signal transduction evoked by novel stimulatory ligands.

30 For therapeutic application, the active compound (amylin, adrenomedullin, analog or ligand) will be formulated as a medicament. The details of the formulation will ultimately depend upon the active compound itself and upon the route of administration chosen. It will however be usual for the medicament to include combination of the active compound with a suitable carrier, vehicle or diluent.



Dosage rates will also be active compound and administration route dependent. However, by way of example, the dosage of active compound to be administered by injection will be in the range of 0.01-100 mg/kg of body weight.

- 5 Further, while formulations in which the active compounds represent the sole active principle are most likely to be used, it is by no means intended that formulations which are suitable for combination therapies be excluded. The active compound can be administered together with any other therapeutic agent, including any other agent which has an effect on chondrocyte proliferation.

- 10 The invention, in its various aspects, will now be illustrated by the experimental section which follows. It will however be appreciated that the experiments are non-limiting.

15 **EXPERIMENTAL**

**METHODS**

**(a) Chondrocyte Monolayer Cell Cultures**

- 20 Fresh cartilage samples were collected from the tibial plateaus and femoral condyles of mature, healthy crossbred dogs (2-4 years; 20-25 kg). The chondrocytes were isolated as previously described (Connective Tissue Research 1988; 18:205-222). Briefly, the chondrocytes were obtained by pronase and collagenase digestion of the cartilage, then the cells were centrifuged, washed and resuspended in media before  
25 being cultured in 75 cm<sup>2</sup> tissue culture flasks. The cells were incubated under 5% CO<sub>2</sub> and 95% air at 37°C. Confluence was reached by 7-10 days, at which time the cells were subcultured. After trypsinization, the cells are rinsed and resuspended in fresh medium, then seeded at 5 x 10<sup>4</sup> cells/ml in 24-well plates (0.5 ml cell suspension per well, ie. 1.4 x 10<sup>4</sup> cells/cm<sup>2</sup>). *Proliferation studies* (cell counts and  
30 thymidine incorporation) were performed. Subconfluent population were changed to serum-free medium with 0.1% bovine serum albumin plus the experimental compounds. Cell numbers were analysed at 24 hours after addition of the peptide or vehicle. The cell numbers were determined using a haemocytometer chamber. Results were expressed per well. *[<sup>3</sup>H]-thymidine incorporation* was assessed by  
35 pulsing the cells with [<sup>3</sup>H]-thymidine (1uCi/well) two hours before the end of the experimental incubation. The experiment was terminated at 24 hours by washing

the cells in media containing cold thymidine followed by the addition of 10% trichloroacetic acid. The precipitate was washed twice with ethanol:ether (3:1) and the wells desiccated at room temperature. The residue was redissolved in 2 M KOH at 55°C for 30 mins, neutralised with 1 M HCl, and an aliquot counted for radioactivity. Results were expressed as dpm per well. For both cell counts and thymidine incorporation, each experiment was performed at least 4 different times using experimental groups consisting of at least 6 wells.

**(b) Chondrocytes 3-dimensional cell cultures in alginate beads**

Alginate head cultures were established as described by Guo, *et al.* Culture and growth characteristics of chondrocytes encapsulated in alginated beads. *Connective Tissue Research* 19:277-297 (1989). Briefly the cells were suspended in a solution of 1.25% (wt/vol) alginate in HEPES (20 mM HEPES buffer pH neutral) at a density of  $2 \times 10^6$  cells/ml. The suspension of chondrocytes were slowly extruded through a 22-gauge needle in a dropwise manner into 40 ml of 0.1 M  $\text{CaCl}_2$  solution. After instantaneous gelation, the beads were allowed to further polymerise in  $\text{CaCl}_2$  solution (10 mins, room temperature). The beads were washed sequentially, twice in 0.15 M NaCl and twice in Dulbecco's modified Eagle's medium (DME). After the washing procedure, the beads were placed into 24-well culture plates (10 beads/well) and fed with 1ml 10% fetal calf serum (FCS) SMW with 5µg/ml ascorbic acid. The cultures were maintained at 37°C in a humidified atmosphere of 5%  $\text{CO}_2$  in air. The medium was changed every second day. On day 4 and 6 of culture, peptide or vehicle was added. Cell numbers were analysed at day 8 by exposing alginate beads to 50 mM ethylenediaminetetraacetic acid (EDTA) for approximately 10 minutes at 37°C. Counting was performed in a haemocytometer chamber. Results were expressed per well. Tritiated-thymidine incorporation ( $^3\text{H}$ -thymidine) was assessed by pulsing the beads with  $^3\text{H}$ -thymidine (1µCi/well) 48 hours before the end of experimental incubation. Experiments were then terminated at day 8 of culture by dissolving the beads in 50 mM EDTA. The cells were washed twice with distilled water by centrifuging. Pellets were resuspended and counted for radioactivity.

**(c) In Vivo Study: Experimental Design**

Two groups of 20 sexually mature male Swiss mice aged between 40 and 50 days and weighing 25-32g, were given daily subcutaneous injections (50 ul) in the loose skin at the nape of the neck for 5 days/week over 4 consecutive weeks. The treated

group was injected with peptide at a dose of 300 ug/kg/injection and the control group was injected with vehicle (water). Animals were housed in a room maintained at 20°C on 12-hour light/dark cycles. They were fed diet 86 rodent pellets (New Zealand Stockfeed Ltd) ad libitum throughout the experiment. Each animal's weight was recorded at the beginning and end of the experiment. One day after the last injection, animals were sacrificed by cervical dislocation. They study had the approval of the local institutional review board.

The tibiae were dissected free of adherent tissue. Tibial lengths were recorded by measuring the distance between the proximal epiphysis and the distal tibio-fibular junction using an electronic micrometer (Digimatic Calipers, Mitutoyo, Japan). Bones were placed in 10% phosphate-buffered formalin for 24 hours and then dehydrated in a graded series of ethanol solutions and embedded, undecalcified, in methylmethacrylate resin. Tibiae were sectioned longitudinally through the frontal plane and calvariae were cut cross-sectionally at the base of the parietal bone. All sections were 4 um thick and were cut on a Leitz microtome using a tungsten-carbide knife (Microknife Sharpening, Utah, USA). Sections were mounted on gelatin-coated slides and air-dried. They were stained with Goldner's tri-chrome and examined using an Olympus BX 50 microscope (Olympus Optical Co Ltd, Tokyo, Japan) which was attached to an Osteomeasure Image Analyzer (Osteometrics Inc. Atlanta, GA).

Tibial histomorphometric analyses were made from three adjacent sections one third of the way through the anterior/posterior depth of the proximal tibiae. Epiphyseal growth plate thickness was measured at three sites evenly spaced along its length. All measurements were made by one operator who was blinded to the treatment group of each bone.

### Materials

Rat amylin was sourced from Bachem California, Torrance, CA, USA. Lyophilised material was dissolved in water prior to administration. Methylmethacrylate was purchased from Acros Organics N.V., Geel, Belgium.

Rat amylin-(1-8) used in this study was a COOH-terminal amide synthesized on methylbenzhydrylamine resin by standard solid-phase techniques followed by hydrogen fluoride deprotection and cleavage from the resin. Amylin-(1-8) was

cyclized in a dilute solution of 90% acetic acid by treatment with methanol solutions of iodine and purified to >96% homogeneity by reverse-phase high performance liquid chromatograph (RP HPLC). Structures were confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF system, model G2025 A, Hewlett Packard CA, USA) and amino acid analysis of acid hydrolysates.

Human adrenomedullin and its fragments were synthesized on methylbenzhydrylamine resin using standard solid-phase procedures, and cleaved with hydrogen fluoride/anisole. Sequences containing a disulfide bridge were cyclized by titration with  $I_2$  in 90% acetic acid/water solutions. Crude materials were purified by gel filtration on Sephadex columns in 50% acetic acid followed by gradient elution on C18 silica using acetonitrile/0.1% trifluoroacetic acid eluants. Homogeneity of final peptides was assessed by thin layer chromatography, analytical HPLC, amino acid analysis and matrix-assisted laser-desorption-ionization mass spectroscopy. Purities were usually >98%.

### Statistical Analysis

Data are presented as mean  $\pm$  sem. Where parameters have been measured more than once in each animal these values have been averaged to produce a single value for each animal before further analysis. The significant of treatment effects was evaluated using Student's *t* tests for unpaired data. These comparisons were specified a priori, so adjustment of  $\alpha(0.05)$  was not necessary.

## RESULTS

### Amylin

#### (a) Chondrocyte Cell Cultures

Amylin influenced chondrocyte proliferation, increasing cell numbers from  $4.12 \pm 0.23 \times 10^4$  (mean  $\pm$  sem) in control cells to  $5.11 \pm 0.21 \times 10^4$  in those cells incubated with amylin ( $p=0.01$ ) as well as increasing thymidine incorporation (ie. DNA synthesis) from  $20725 \pm 997$  dpm in control cells to  $25937 \pm 1203$  dpm in amylin-treated cells.

(b) **Chondrocytes 3-dimensional cell cultures in alginate beads**

Amylin again influenced chondrocyte proliferation, increasing cell numbers from  $5.58 \pm 0.16 (x10^4)$  (mean  $\pm$  sem) in control cells to  $6.07 \pm 0.05 (x10^4)$  in those cells incubated with amylin ( $10^{-10}M$ ) ( $p < 0.03$ ) as well as increasing thymidine incorporation (ie. DNA synthesis) from  $1135 \pm 85$  dpm in control cells to  $2584 \pm 229$  dpm in amylin-treated cells ( $p < 0.001$ ).

(c) **In Vivo Study**

Amylin influenced the tibial growth plate, increasing its width from  $0.083 \pm 0.005$  mm (mean  $\pm$  sem) in the control animals to  $0.108 \pm 0.003$  mm in those receiving amylin ( $P = 0.0002$ ) (Figure 1). The total length of the tibiae was also increased from  $11.31 \pm 0.07$  mm in control animals to  $11.67 \pm 0.09$  mm in animals injected with amylin ( $P = 0.004$ ) (Figure 2).

15 **Amylin 1-8**

(a) Amylin-(1-8) also influenced chondrocyte proliferation, increasing cell numbers from  $3.23 \pm 0.11 (x10^4)$  (mean  $\pm$  sem) in control cells to  $3.63 \pm 0.09 (x10^4)$  in those cells incubated with amylin-(1-8) ( $10^{-8}M$ ) ( $p = 0.02$ ) as well as increasing thymidine incorporation (DNA synthesis) from  $26859 \pm 423$  dpm in control cells to  $28932 \pm 628$  dpm in amylin-(1-8) treated cells ( $p = 0.02$ ).

(c) The growth plate width in the proximal tibiae of mice injected systemically with amylin-(1-8) is significantly increased compared to control animals (mean  $\pm$  sem:  $0.111$  mm  $\pm$   $0.004$  compared to  $0.081$  mm  $\pm$   $0.004$ ;  $p < 0.0001$ ). See Figure 3.

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**Adrenomedullin**

(a) Adrenomedullin influenced chondrocyte proliferation, increasing cell numbers from  $1.79 \pm 0.07 (x 10^4)$  (mean  $\pm$  sem) in control cells to  $2.27 \pm 0.12 (x 10^4)$  in those cells incubated with adrenomedullin ( $10^{-9}M$ ) ( $p < 0.01$ ).

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**Adrenomedullin-(27-52)**

(c) Adrenomedullin-(27-52) increased the growth plate width from  $0.094$  mm  $\pm$   $0.003$  (mean  $\pm$  sem) in control animals to  $0.11$  mm  $\pm$   $0.003$  in adrenomedullin-(27-52) ( $p = 0.003$ ). See Figure 4.

## INDUSTRIAL APPLICATION

The above results clearly show that amylin and its anlogs (amylin-(1-8), for example) has the ability to stimulate chondrocyte proliferation. Similarly, adrenomedullin and its analogs (adrenomedullin-(27-52) have equivalent capability.

The results additionally show the ability of both amylin, adrenomedullin and their analogs to influence the growth of cartilage as well as increased bone growth. This latter effect is consistent with the formation of bone on a template of cartilage tissue.

Both amylin and adrenomedullin are believed to be exerting the effect on chondrocyte proliferation/cartilage growth/bone formation through the mediation of the amylin/adrenomedullin receptor.

The present invention therefore provides new approaches to chondrocyte proliferation. These involve firstly increasing the active concentration of amylin/adrenomedullin in a patient and secondly the activation of the amylin/adrenomedullin receptor localised on chondrocyte cells.

The approaches of the invention have application in the treatment of patients in a variety of conditions. Principal amongst these are conditions where the patient is suffering from a cartilage defect, either through injury or through degenerative, inflammatory or other disease.

The approaches of the invention also have application in the stimulation of bone growth, particularly lineal bone growth. This provides the invention with application in treating patients (for example, children) who are of short stature or who otherwise suffer from defects which would benefit from stimulation of the growth of limb bones.

The invention also has application *in vitro*. Extracted chondrocytes can be proliferated using the present methods. The proliferated chondrocytes can then be employed in methods of therapy, particularly those which involve the treatment of damaged cartilage.

5    appended claims.

Year	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	